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Judge
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	RICHTER, Mark M. *et al.*
Serial No:	09/074,472
Filed:	May 7, 1998
For:	ASSAYS EMPLOYING ELECTROCHEMILUMINESCENT LABELS AND ELECTROCHEMILUMINESCENCE QUENCHERS
Art Unit:	1655
Examiner:	Arun Chakrabarti, Ph.D.

RESPONSE UNDER 37 CFR §1.111

Assistant Commissioner for Patents
Washington, DC 20231

November 20, 2001

Dear Sir:

This reply is in response to the Office Action dated June 6, 2001, which has a shortened statutory period for response of three months that expired September 6, 2001. A petition for a 3-month extension of time until December 6, 2001 is enclosed herewith.

AMENDMENT

Please amend the above-identified patent application as follows:

CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that the following paper is being sent via facsimile to the U.S. Patent and Trademark Office on November 20, 2001

Leah Robison

11/26/2001 FJACKSON 00000004 022958 09074472

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In the Claims:

Please cancel claims 28 and 29 without prejudice.

Please add the following new claims 30-33:

30. (new) A method for qualitative or quantitative electrochemiluminescence detection of an oligonucleotide target analyte in a sample, the method comprising the steps of:

(a) preparing an assay mixture comprising:

the sample,

one or more assay reagents comprising a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe complementary to the analyte and ~~capable of~~ hybridizing therewith, the label ~~capable of~~ generating a detectable electrochemiluminescent emission, wherein the labeled complex is immobilized on a magnetic particle,

an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone, and

a coreactant,

(b) bringing the assay mixture into contact with a working electrode,

(c) applying a potential to the electrode, thereby enabling an electrochemiluminescence reaction to proceed,

(d) separating unhybridized labeled complex from hybridized labeled complex,

(e) measuring the electrochemiluminescent emission produced by the label hybridized to the analyte via the oligonucleotide probe, and

(f) correlating the measured electrochemiluminescent emission with the presence or amount of the analyte in the sample.

31. (new) A method for qualitative or quantitative electrochemiluminescence detection of an oligonucleotide target analyte in a sample, the method comprising the steps of:

(a) preparing an assay mixture comprising:

the sample,

one or more assay reagents comprising a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe, complementary to the analyte and ~~capable of~~ hybridizing therewith, the label ~~capable of~~ generating a detectable electrochemiluminescent emission, the labeled complex further comprising an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone, the quenching moiety attached to the probe, wherein the labeled complex is immobilized on a magnetic particle, and

a coreactant,

(b) bringing the assay mixture into contact with a working electrode,

(c) applying a potential to the electrode, thereby enabling an electrochemiluminescence reaction to proceed,

(d) separating unhybridized labeled complex from hybridized labeled complex,

(e) measuring the electrochemiluminescent emission produced by the label hybridized to the analyte via the oligonucleotide probe, and

(f) correlating the measured electrochemiluminescent emission with the presence or amount of the analyte in the sample.

32. (new) An assay reagent kit for qualitative or quantitative electrochemiluminescence detection of an oligonucleotide target analyte in a sample, the assay reagent kit comprising, in one or more containers in packaged combination:

one or more assay reagents comprising a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe ~~capable of~~ hybridizing with the analyte, the label ~~capable of~~ generating a detectable electrochemiluminescent emission, wherein the labeled complex is immobilized on a magnetic particle,

an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone, and

a coreactant.

33. (new) An assay reagent kit for qualitative or quantitative electrochemiluminescence detection of an oligonucleotide target analyte in a sample, the assay reagent kit comprising, in one or more containers in packaged combination:

one or more assay reagents comprising a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe, complementary to the analyte and capable of hybridizing therewith, the label capable of generating a detectable electrochemiluminescent emission, the labeled complex further comprising an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone, the quenching moiety attached to the probe, wherein the labeled complex is immobilized on a magnetic particle, and

a coreactant.

REMARKS

In view of the comments that follow, and pursuant to 37 CFR §1.111, Applicants respectfully request reconsideration of the Official Action of June 6, 2001.

Status of claims

Claims 30-33 are currently pending in the application. Claims 28 and 29 have been cancelled without prejudice. Method claim 28 has been replaced by new method claims 30-31, and kit claim 29 has been replaced by new kit claims 32-33. Claims 28 and 29 were rejected in the instant office action; however, Applicants' comments below relate to the rejections as applied to the new claims. A clean copy of the newly added claims is attached herewith for the Examiner's convenience.

No new matter has been added. Support for wording not previously recited in claims 28 and 29 is to be found in the specification as follows:

The term "oligonucleotide target" in claim 30, line 2, is found in the specification on page 65, lines 2-3.

The term "one or more assay reagents" in claim 30, line 6, is found in the specification on page 6, line 24.

The term "labeled complex" in claim 30, line 6, is found in the specification on page 58, line 14.

The term "ruthenium bipyridine complexes and osmium bipyridine complexes" in claim 30, line 8, is found in the specification on page 4, lines 25-26.

The term "oligonucleotide probe complimentary to the analyte and capable of hybridizing therewith" in claim 30, lines 9-10, is found in the specification on page 8, lines 8-10 and on page 33, lines 21-22.

The term "label capable of generating a detectable electrochemiluminescent emission" in claim 30, lines 1-11, is found in the specification on page 13, line 24.

The term "labeled complex is immobilized on a magnetic particle" in claim 30, lines 11-12, is found in the specification on page 58, lines 14-17.

The term "phenol and benzoquinone" in claim 30, line 14, is found in the specification on page 58, line 16 (phenol) and on page 61, line 11 (benzoquinone).

The term "coreactant" in claim 30, line 15, is found in the specification on page 47, lines 4-9.

The term "working electrode" in claim 30, line 16, is found in the specification on page 43, lines 27-28.

The term "applying a potential to the electrode" in claim 30, line 17, is found in the specification on page 45, lines 8-9.

The term "separating unhybridized labeled complex from hybridized labeled complex" in claim 30, lines 19-20, is found in the specification on page 8, lines 11-15 and on page 9, lines 9-23.

The term "measuring the electrochemiluminescent emission produced" in claim 30, line 21, is found in the specification on page 6, lines 24-25.

The term "correlating the measured electrochemiluminescent emission with the presence or amount of the analyte in the sample" in claim 30, lines 23-24, is found in the specification on page 6, lines 25-26.

The term "quenching moiety attached to the probe" in claim 31, lines 13-14, is found in the specification on page 61, lines 9-12.

The term "one or more containers in packaged combination" in claim 32, lines 3-4, is found in the specification on page 43, lines 14-19.

Rejections under 35 USC §103 (a) (Examiner's paragraph 4)

Claim 28 has been rejected under 35 USC §103 (a) as being unpatentable over Aizawa *et al.*, *Proceedings Electrochemical Society 93-97*, 662-673, 1993 (hereinafter "Aizawa") in view of U.S. Patent No. 5,925,517 issued July 20, 1999 to Tyagi *et al.* (hereinafter "Tyagi").

The Examiner argues that Aizawa teaches a method for quantitatively detecting an analyte in a sample composition comprising the steps of:

(a) preparing an assay mixture comprising the sample composition and a reagent having an ECL label,

(b) determining any difference between the ECL emissions of the assay mixture prepared in step (a) and an assay mixture comprising the reagent having an ECL label and a known amount of the analyte, and

(c) correlating any difference determined in step (b) with the amount of analyte in the sample.

Further, Aizawa teaches a method wherein the ECL label comprises a polyaromatic hydrocarbon, and Aizawa teaches a method wherein the ECL label comprises ruthenium or osmium.

Even further, Aizawa teaches the method wherein the analyte comprises an oligonucleotide, polypeptide, antibody, antigen, and enzyme, an enzyme substrate and polysaccharide. And finally, Aizawa teaches a method wherein the known amount of analyte is zero.

Aizawa does not teach a method wherein reagent having an ECL quenching moiety, the quenching moiety comprising at least one benzene moiety, the ECL quenching moiety comprises at least one moiety selected from the group consisting of phenol moieties, quinone moieties, benzene carboxylic acid moieties, and benzene carboxylate moieties.

Further, Aizawa does not teach a method wherein the reagent having an ECL label and the reagent having an ECL quenching moiety are either the same or different reagent.

Tyagi teaches a method wherein the quenching moiety comprising at least one phenol moiety, at least one benzene carboxylic acid moiety or at least one benzene carboxylate moiety. Fluorescein, the quenching label, has at least two benzene moieties.

The Examiner's position is that it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to include the group of chemicals containing substituted benzene rings of Tyagi in the method of Aizawa since

Tyagi states, "By using multiple probes with interactive labels that generate different, non-interfering detectable signals, e.g., fluorescence at different wavelengths or fluorescence and colored product formation, assays of this invention can detect multiple targets in a single assay." An ordinary practitioner would have been motivated to combine and compare the electrochemiluminescence quenching chemicals containing deferentially substituted benzene ring of Tyagi into the method of Aizawa in order to achieve the express advantages, as noted by Tyagi, of electrochemiluminescence quenching chemicals which provide detection of multiple targets in a single assay.

Applicants argue that the examiner's case for *prima facie* obviousness has not been made. The combination of Aizawa and Tyagi together do not produce Applicants invention of a method or test kit for electrochemiluminescent detection of an analyte using an ECL labeled probe and an ECL quenching moiety, wherein the labeled probe is immobilized on a magnetic particle.

Aizawa teaches two types of biosensor detection methods based on electrochemiluminescence. The first is a homogeneous immunoassay in which an electrochemiluminescent label, luminol, is used. The analyte is an antigen (human immunoglobulin G). When an antibody to immunoglobulin G, which has been bound to luminol, forms an immunocomplex with antigen, quenching of the electrochemiluminescence signal from luminol is observed. The amount of signal quenching can be correlated to the amount of immunoglobulin G in the sample.

The second method taught is a method for screening DNA intercalators using electrochemiluminescent sensing. An intercalator to be screened, e.g., cisplatin or actinomycin D, is combined with double-stranded DNA, followed by addition of a ruthenium-bathophenanthroline complex (which also interacts with DNA), and the resulting luminescence compared to drug concentration. The intercalated ruthenium complex emits no electrochemiluminescence. However, binding of the ruthenium complex to DNA is inhibited by binding between the drug and the DNA.

Aizawa does not teach a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe. Aizawa does not teach an electrochemiluminescent quenching moiety selected from phenol and benzoquinone. Aizawa also fails to teach the immobilization of such a labeled complex on a magnetic particle. Furthermore, Aizawa does not teach a labeled complex comprising an electrochemiluminescent label selected from ruthenium bipyridine complexes and osmium bipyridine complexes attached to a nucleotide probe and a quenching moiety attached to the probe. Aizawa also fails to teach the immobilization of such a labeled complex on a magnetic particle. Finally, Aizawa fails to teach hybridization of a probe to a target nucleotide sequence.

Tyagi teaches the use of an oligonucleotide probe in a homogeneous assay, the probe possessing both a fluorophore label and a fluorescence quencher (an "affinity pair"). In the absence of target oligonucleotide in a sample, portions of the probe hybridize with itself, bringing the fluorophore and the fluorescence quencher into close proximity. In this form, no fluorescence signal is observed. In the presence of target oligonucleotide, the oligonucleotide probe dehybridizes and preferentially hybridizes with the target oligonucleotide, and in so doing, separates the fluorophore and the quencher, and in this form, a fluorescence signal is observed.

Tyagi does not teach an electrochemiluminescent method nor an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone. Tyagi also fails to teach a label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes. Tyagi suggests (column 16, lines 23-27) that luminescent label moieties can be paired with appropriate quenching moieties and can be selected from categories including an electrochemiluminescent label. However, Tyagi does not teach any specific label-quencher pairs that are operable, nor does he suggest how one might choose operable electrochemiluminescent label-quencher pairs that would be compatible with nucleic acid

hybridization. One skilled in the art to which the present invention belongs would not assume that electrochemiluminescent quenching pairs would behave similarly to fluorescence quenching pairs, especially in such sensitive assays as those involving nucleic acid hybridization. Applicants teach in their specification on page 12, lines 1-7: "Unlike the quenching of ECL, the quenching of photoluminescence has been widely studied, and many compounds are known to quench photoluminescence under a variety of conditions. In sharp contrast, only a few compounds are known to efficiently quench ECL, and many of those which are well known (e.g., methylviologen carboxylate) either only poorly quench ECL or are impractical for use in assays."

If the skilled artisan attempted to combine the method of Aizawa with the method of Tyagi, he would not arrive at the method of the present invention. Aizawa teaches an antigen analyte and an antibody that binds thereto. Aizawa also teaches a bathophenanthroline-ruthenium complex that depends upon interaction with DNA for signal quenching. Tyagi teaches a nucleotide analyte and a nucleotide probe that binds thereto. Aizawa teaches no external quencher but relies instead upon a quenching effect caused by immunocomplexation. Tyagi teaches an external fluorescence quencher. There is no suggestion in either Aizawa or Tiyagi how to combine the features to provide a method for electrochemiluminescence detection of an oligonucleotide target analyte. As is demonstrated in Applicants' specification, known electrochemiluminescence quenchers sometimes provide surprising results. For example, in Example 5 (pages 52 and 53), it was demonstrated that the effect of phenol on fluorescence was much less than the effect on electrochemiluminescence, and the effect on photoluminescence of increasing concentrations of phenol was exactly opposite the effect of increasing concentrations of phenol on chemiluminescence. And in Example 9 (page 55), an increase in photoluminescence was observed the addition of hydroquinone to a solution containing a ruthenium luminophore, while the opposite effect was observed for electrochemiluminescence. However, the addition of catechol and also benzoquinone to a luminophore produced decreases in photoluminescence.

Furthermore, neither Aizawa nor Tiyagi teach or suggest a heterogeneous assay requiring a separation step. In fact, Tiyagi actually teaches away from such an assay at column 6, lines 38-40.

Based upon the present amendments and the foregoing remarks, Applicants argue that the case of *prima facie* obviousness has not been made. The Examiner's reconsideration of the rejection under 35 USC §103 (a) as now applied to new claims 30 and 31 is respectfully requested.

Rejections under 35 USC §103 (a) (Examiner's paragraph 5)

Claims 28-29 have been rejected under 35 USC §103 (a) as being unpatentable over Aizawa in view of Tyagi and further in view of Stratagene Catalog, p. 39, 1988 (hereinafter "Stratagene").

The Examiner argues that Aizawa in view of Tyagi expressly teaches the method claims and assay reagents of claim 28 as described above in detail. Aizawa in view of Tyagi does not teach the motivation to combine all the reagents for detecting an analyte in a sample in the form of a kit.

Stratagene teaches a motivation to combine reagents into a kit format.

The Examiner's position is that it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine a suitable container, ECL label and ECL quenching moiety of Aizawa in view of Tyagi into a kit format as discussed by Stratagene since Stratagene teaches a motivation for combining reagents for use in an assay into a kit.

Applicants argue that even the combination of all three references still does not make the claimed invention and that the examiner's case of *prima facie* obviousness has not been made. As argued above, the combination of Aizawa and Tyagi together do not produce Applicants invention of a method for electrochemiluminescent detection of an

analyte using an ECL labeled probe and an ECL quenching moiety, wherein the labeled probe is immobilized on a magnetic particle. The Stratagene reference does not provide the additional teaching necessary to arrive at the invention of Applicants' reagent kit.

Based upon the present amendment and the foregoing remarks, Applicants argue that the case of *prima facie* obviousness has not been made. The Examiner's reconsideration of the rejection under 35 USC §103 (a) as now applied to new claims 32 and 33 is respectfully requested.

* * * * * *

Applicants submit that their application is now in condition for allowance, and entry of the present amendment and favorable reconsideration of their application in light of the above remarks is respectfully requested. Allowance of claims 30-33 at an early date is earnestly solicited.

The Examiner is hereby authorized to charge any fees associated with this amendment to Deposit Account No. 02-2958. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

November 20, 2001

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analyte using an ECL labeled probe and an ECL quenching moiety, wherein the labeled probe is immobilized on a magnetic particle. The Stratagene reference does not provide the additional teaching necessary to arrive at the invention of Applicants' reagent kit.

Based upon the present amendment and the foregoing remarks, Applicants argue that the case of *prima facie* obviousness has not been made. The Examiner's reconsideration of the rejection under 35 USC §103 (a) as now applied to new claims 32 and 33 is respectfully requested.

* * * * * *

Applicants submit that their application is now in condition for allowance, and entry of the present amendment and favorable reconsideration of their application in light of the above remarks is respectfully requested. Allowance of claims 30-33 at an early date is earnestly solicited.

The Examiner is hereby authorized to charge any fees associated with this amendment to Deposit Account No. 02-2958. A duplicate copy of this sheet is enclosed.

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30. A method for qualitative or quantitative electrochemiluminescence detection of an oligonucleotide target analyte in a sample, the method comprising the steps of:

(a) preparing an assay mixture comprising:

the sample,

one or more assay reagents comprising a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe complimentary to the analyte and capable of hybridizing therewith, the label capable of generating a detectable electrochemiluminescent emission, wherein the labeled complex is immobilized on a magnetic particle,

an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone, and

a coreactant,

(b) bringing the assay mixture into contact with a working electrode,

(c) applying a potential to the electrode, thereby enabling an electrochemiluminescence reaction to proceed,

(d) separating unhybridized labeled complex from hybridized labeled complex,

(e) measuring the electrochemiluminescent emission produced by the label hybridized to the analyte via the oligonucleotide probe, and

(f) correlating the measured electrochemiluminescent emission with the presence or amount of the analyte in the sample.

31. A method for qualitative or quantitative electrochemiluminescence detection of an oligonucleotide target analyte in a sample, the method comprising the steps of:

(a) preparing an assay mixture comprising:

the sample,

one or more assay reagents comprising a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe, complimentary to the analyte and capable of hybridizing therewith, the label capable of generating a detectable electrochemiluminescent emission, the labeled complex further comprising an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone, the quenching moiety attached to the probe, wherein the labeled complex is immobilized on a magnetic particle, and

a coreactant,

(b) bringing the assay mixture into contact with a working electrode,

(c) applying a potential to the electrode, thereby enabling an electrochemiluminescence reaction to proceed,

(d) separating unhybridized labeled complex from hybridized labeled complex,

(e) measuring the electrochemiluminescent emission produced by the label hybridized to the analyte via the oligonucleotide probe, and

(f) correlating the measured electrochemiluminescent emission with the presence or amount of the analyte in the sample.

32. An assay reagent kit for qualitative or quantitative electrochemiluminescence detection of an oligonucleotide target analyte in a sample, the assay reagent kit comprising, in one or more containers in packaged combination:

one or more assay reagents comprising a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe capable of hybridizing with the analyte, the label capable of generating a detectable electrochemiluminescent emission, wherein the labeled complex is immobilized on a magnetic particle,

an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone, and

a coreactant.

33. An assay reagent kit for qualitative or quantitative electrochemiluminescence detection of an oligonucleotide target analyte in a sample, the assay reagent kit comprising, in one or more containers in packaged combination:

one or more assay reagents comprising a labeled complex comprising an electrochemiluminescent label selected from the group consisting of ruthenium bipyridine complexes and osmium bipyridine complexes attached to an oligonucleotide probe, complimentary to the analyte and capable of hybridizing therewith, the label capable of generating a detectable electrochemiluminescent emission, the labeled complex further comprising an electrochemiluminescence quenching moiety selected from the group consisting of phenol and benzoquinone, the quenching moiety attached to the probe, wherein the labeled complex is immobilized on a magnetic particle, and

a coreactant.